Salmonella Recovery from Tomato Fruit Surfaces as Affected by Ethylene

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SUMMARY. The effect of ethylene on the survival of *Salmonella* cells residing on unwounded surfaces of tomato (*Solanum lycopersicum*) fruit was investigated in this study. Inoculated fruit were stored in flow-through chambers that were adjusted to maintain an environment simulating a tomato ripening room. Fruit were held at 20 °C and ≥95% relative humidity after surface inoculation with the low virulence and rifamycin-resistant pathogen *S. enterica* ssp. *enterica* serovar Typhimurium strain LT2 (*S. Typhimurium*). Tomato fruit were treated either with a continuous flow (rate, ≈200 mL·min⁻¹) of air or with ≥150 µL·L⁻¹ ethylene in air. Bacterial recovery at 0, 24, 48, and 72 h after initiation of treatment showed that total populations of the *S. Typhimurium* cells declined in both the air and ethylene treatments during the first 24 to 48 h of storage, then increased to near initial levels by 72 h, similar to decline and recovery reported by other researchers in nonethylene treatment trials. These results suggest that although *Salmonella* can survive on the surfaces of tomato fruit in typical ripening rooms, proliferation of *Salmonella* is neither promoted nor inhibited by ethylene exposure.

Recently, the consumption of raw tomatoes has been indicated in several outbreaks of enteric disease incited by serovars of the bacteria *Salmonella enterica* ssp. *enterica* (Cummings et al., 2001; Hedberg et al., 1999; Toth et al., 2002; Wood et al., 1991). In most cases, diced or otherwise foodservice-prepared tomatoes were responsible. Contamination of the fruit has been traced back to packinghouses in some cases, but the specifics of contamination and the reliability of these “trace-backs” have not been determined (Centers for Disease Control and Prevention, 2005).

In packinghouses, after being graded and packed, mature green tomatoes are typically treated with the gaseous plant growth regulator ethylene (C₂H₄) for a number of days at 20 to 21 °C in ripening rooms. It has been recommended that this treatment need last no more than 3 d (Sargent et al., 2005), but as many as 9 d of treatment have been reported by industry professionals (authors’ pers. obs.), depending on the maturity of the fruit at harvest. Certain fungi have been observed to react to

the presence of C₂H₄ with stimulated growth or increased germ tube elongation (El-Kazzaz et al., 1983). Bacterial response to C₂H₄, however, has not been characterized. Specifically, a response by *Salmonella* to elevated C₂H₄ concentrations has not been reported. If cells of *Salmonella* are responsive to C₂H₄, fruit ripening treatments may lead to greater survival or proliferation of any *Salmonella* present, potentially increasing the likelihood of disease outbreak in consumers. Previous work suggests that *Salmonella* cells in ripening room conditions [≈20 °C and 85% to 90% or higher relative humidity (RH)] can survive and multiply for at least 7 d (Zhuang et al., 1995), possibly surviving for more than 28 d (Allen, 2003). A 30% reduction in RH values (from 90% to 60%) at 20 °C reduced survival (Allen, 2003; Guo et al., 2002). However, the presence and any effects of C₂H₄ in those experiments were not explored. The research reported in

this paper was performed to determine the effect, if any, of C₂H₄ in simulated ripening room conditions on the survival of *Salmonella* on the surfaces of whole tomato fruit.

Materials and methods

Tomato fruit were acquired from Florida growers (cultivars either unknown or Florida-47) and stored in the laboratory at room temperature (22 °C) to expedite ripening or in a reach-in chamber at 12.5 °C to stall ripening, for up to 5 d before use. Fruit were randomized by ripeness stage according to a commercial ripeness guide (United Fresh Fruit and Vegetable Association and U.S. Department of Agriculture, 1975), ranging from breaker to red, as they were grouped for each trial. Fruit were collected before being run along grading lines and so were unwashed and did not have any coatings (wax or mineral oils, etc.) applied.

A stock culture of the low-virulence pathogen *S. enterica* ssp. *enterica* serovar Typhimurium, strain LT2 (ATCC 15277), that was adapted for resistance to rifamycin (Rif^r at 200 µg·L⁻¹) was used as the test inoculum. Cells were recovered from Protect Bacterial Preserver Beads (Scientific Device Laboratories, Des Plaines, Ill.) by incubation in tryptic soy broth (TSB). After overnight growth (12 h) on a rotary shaker (30 rpm) in an incubator (40 °C), cells were reinoculated to fresh TSB and allowed to incubate overnight again under the same conditions. This was done a total of three times. After the third generation, cells were streaked with tryptic soy agar (TSA) with rifamycin SV sodium salt (Sigma Chemical Co., St. Louis, Mo.) at 80 µg·L⁻¹ and stored overnight at 40 °C. Antibiotic concentrations were gradually increased on subsequent generations in this manner until surviving colonies were present on agar plates with 200 µg·L⁻¹ rifamycin. Cells were

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	µL	3.3814×10^{-5}
29.5735	fl oz	mL	0.0338
0.0283	ft ³	m ³	35.3147
0.4536	lb	kg	2.2046
1	ppb	µg·L ⁻¹	1
1	ppm	µL·L ⁻¹	1
$(^{\circ}\text{F} - 32) \div 1.8$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$(1.8 \times ^{\circ}\text{C}) + 32$

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then maintained on 200 $\mu\text{g}\cdot\text{L}^{-1}$ Rif^r TSA plates at room temperature.

For inoculation, a sample of the prepared rifamycin-resistant cells was transferred to a 200- $\mu\text{g}\cdot\text{L}^{-1}$ Rif^r TSB tube and incubated overnight at 40 °C on a rotary shaker. The suspension was spun down in a centrifuge (IEC Centra MP4R; International Equipment Co., Needham Heights, Mass.) at 450 g_n for 10 min. The supernatant was decanted and the pellet resuspended in 10 mL phosphate buffered saline, then centrifuged again. Cells were washed using this procedure three times before testing. A sample of the stock inoculum was serially diluted and plated using pour-plate technique and 100 $\mu\text{g}\cdot\text{L}^{-1}$ Rif^r TSA for determination of stock concentration. Just before initiation of the trials, each tomato was spot-inoculated 10 times around the blossom scar, across replicates, with 10 μL 7.69 $\log_{10}$ cfu $\cdot\text{mL}^{-1}$ stock inoculum in each spot (100 μL per fruit, or total $\approx 6 \log_{10}$ cfu/fruit). Fruit were allowed to air dry for about 1 h before being placed into a treatment chamber, making sure inoculation points did not contact adjacent fruit or the chamber wall.

C₂H₄ treatment chambers (10-L glass jars) contained a similar ratio of fruit to volume at the same temperature and RH as would be found in a commercial tomato ripening room that was operated using recommended conditions (Sargent et al., 2005). A standard tomato ripening room has space for two truckloads of packed and palletized tomato fruit. A single truckload consists of 20 pallets, with each pallet made up of 100 25-lb boxes of tomatoes. Therefore, a standard two-truckload ripening room containing 40 pallets total includes about 100,000 lb of fruit. A standard ripening room volume is 9980 ft³ (Sherman and Talbot, 1986), so when filled with 100,000 lb fruit, there are ≈ 10 lb fruit/ft³. The volume of the glass jars used as treatment chambers was 10.0 L (0.35 ft³); therefore, 10 fruit [≈ 1.6 kg (3.53 lb)] were placed into each jar. In the third trial (only), five fruit were used for each chamber because of limited resources.

Treatment of tomato fruit consisted of four predetermined time intervals of continuous exposure either to air or to C₂H₄ at $\geq 150 \mu\text{L}\cdot\text{L}^{-1}$, the

recommended dosage of C₂H₄ in a tomato ripening room (Sargent et al., 2005). Each treatment chamber was connected to a flow-through gas system in which C₂H₄ and air from pressurized gas cylinders were mixed at constant pressure via a gas mixing board equipped with needle valve flow meters. Treatment levels were quickly established at the onset of treatment by injecting a calculated volume of pure C₂H₄ into the container. A gas chromatograph (model 540; Tracor Instruments, Austin, Texas) with a photoionization detector and 3-ft stainless steel column packed with alumina F-1, 80/100 size mesh was used to quantify C₂H₄.

The total flow rate to each jar was $\approx 200 \text{ mL}\cdot\text{min}^{-1}$, which exceeded the recommended minimum air exchange in a tomato ripening room of at least one room volume every 6 h (Sargent et al., 2005). To maintain the recommended RH of $\geq 85\%$, deionized water was misted into each chamber before the addition of fruit. Also, during treatment, the gas mixtures were bubbled through deionized water using submerged aquarium aerator stones in separate, in-line, sealed glass jars (500 mL). Treatment jars were exhausted to outside the storage facility via a PVC exhaust system that pulled a slight vacuum, using the Venturi effect. The entire system was held in a walk-in incubator with a regulated temperature (20 °C). To verify the temperature and RH, a digital environmental recorder (WatchDog model 250; Spectrum Technologies, Inc., Plainfield, Ill.) was placed in each of the 72-h treatment chambers (with and without C₂H₄) for all trials.

In each trial, six treatment chambers of tomato fruit were used for two treatments (with and without C₂H₄) and three time periods each (24, 48, and 72 h). A fourth group of fruit was inoculated at the same time as the other treatments (T₀), but was not stored and was included as an untreated control; fruit from this treatment were treated to recover surface inoculated *S. Typhimurium* immediately after the storage trials were initiated. After each predetermined C₂H₄ exposure period had elapsed (including T₀ controls), fruit were removed from one C₂H₄-treated and one untreated chamber and placed on trays. Fruit were immedi-

ately treated to recover any Rif^r-resistant *S. Typhimurium* present on the surface by individually placing fruit into a sealed stomacher bag with 100 $\pm$ 5.0 mL sterile tap water (second trial) or phosphate buffered saline (all other trials) followed by manual rubbing and shaking for 1 min (Burnett and Beuchat, 2001). After manual agitation, the fruit were removed from the bag and discarded, saving the liquid contents and any liberated, suspended cells. Recovery treatment alternated individually between the “with” and the “without” C₂H₄ samples at each sampling time, until recovery treatment had been conducted for all fruit.

After all fruit were treated for bacterial cell recovery and discarded, the liquid samples were each serially diluted three times, at a 1:10 ratio each, in fresh volumes of the same type of liquid used during the recovery suspension. The total dilutions were 10⁻² for the initial 100-mL recovery, 10⁻³ for the first 1:10 dilution, 10⁻⁴ for the second and 10⁻⁵ for the third. A 1-mL sample from each dilution in the series was pour-plated to a 100 $\mu\text{g}\cdot\text{L}^{-1}$ Rif^r TSA Petri dish. Plates were labeled, allowed to solidify, and were then incubated upside down for 48 h in a 37 °C chamber. Plates were stored in loosely sealed 7.0-L anaerobic gas chambers (AnaeroPack System; Mitsubishi Gas Chemical Co., Tokyo, Japan) to avoid desiccation. After incubation, plates were counted for total colony forming units of Rif^r-resistant cells recovered. Plates with 30 to 300 cfu/plate were back-calculated to total cells per fruit, accounting for all dilutions. Data from three trials were averaged and SDs were calculated to determine 95% confidence intervals.

Results and discussion

Recorded C₂H₄ levels were maintained at or above 150 $\mu\text{L}\cdot\text{L}^{-1}$ (the recommended ripening room dose) throughout the experiments. As verified by the digital environmental recorders, the temperature was maintained at 20 $\pm$ 0.6 °C throughout the study and the RH levels in all chambers were $\geq 90\%$ within 2 h, reaching and maintaining more than 99% in less than 12 h. Infusing the treatment chambers with misted water before the addition of fruit ensured the RH was high ($>80\%$) at

Fig. 1. Recovery of Rif-resistant *Salmonella enterica* ssp. *enterica* serovar Typhimurium strain LT2 (*S. Typhimurium*) from intact tomato fruit surfaces, after flow-through treatment with air or $\geq 150 \mu\text{L}\cdot\text{L}^{-1}$ (ppm) ethylene over four time periods ($P = 0.05$).

the initiation of each trial. Use of aquarium aerator stones in the humidifier chambers produced fine bubbles of incoming gas, thus maximizing the total surface area of the gas that was in contact with water and the amount of water vapor carried into the treatment jars. This helped maintain $\geq 97\%$ RH, eliminating loss of bacteria as a result of desiccation (Guo et al., 2002), as was observed in an initial trial without the in-line humidification chambers, when RH remained less than 75% (data not shown).

Inoculum was present and recoverable from all fruit. Data are the compilation results of three trials (Fig. 1) at each treatment time. Error bars represent the 95% confidence interval of recoverable and countable (30–300 cfu/plate) cells. Inoculum recovered from control fruit, immediately after drying and before any C_2H_4 treatment, was $6.2 \log_{10}$ (SD = 0.97) after inoculation with $100 \mu\text{L}$ from a broth of $7.69 \log_{10}$ *S. Typhimurium* (SD = 0.18). After 24 h, $\approx 4.7 \log_{10}$ bacteria were recovered from both treated and untreated fruit, which is a 1.5 log cycle reduction in bacterial populations compared with positive control fruit at T_0 . Bacterial populations recovered after 48 h, with or without C_2H_4 treatment, were not significantly dif-

ferent ($P = 0.05$) from those recovered after 24 h. After 72 h, bacteria that were in C_2H_4 treatments increased to $5.1 \log_{10}$, which was not significantly greater than recovery levels after 24 or 48 h, nor was it different from fruit not treated with C_2H_4 at 72 h. Conversely, fruit treated with air only had an unexpected and significant increase in recoverable cells at 72 h. Similar variations have been previously reported (Allen, 2003), but were not significant in the overall trend of decreasing recovery. Because recovery was not different from C_2H_4 -treated fruit at this time, although the cause of this growth was unknown, it can be surmised that it was not the C_2H_4 treatment.

At each sampling time (Fig. 1), lack of significant differences between treatments ($P = 0.05$) suggests that, at the concentration used in tomato ripening rooms, C_2H_4 does not have an effect upon growth rates of *S. Typhimurium* cells. Although higher C_2H_4 concentrations than those used in this research may have an effect on bacterial recovery, this possibility was not explored. Higher C_2H_4 concentrations are unlikely to be encountered, considering that concentrations used here equal or exceed current recommendations for a tomato ripening room (Sargent et al., 2005). Because it has previously

been shown that *Salmonella* spp. cells can survive on intact tomato fruit surfaces for at least 28 d at the recommended ripening conditions of 20°C and 90% RH (Allen, 2003), it may be of interest to determine whether and how C_2H_4 treatment affects recovery rates of surface-inoculated tomatoes after extended exposure to C_2H_4 (up to or beyond 7 d), or whether C_2H_4 treatment affects subsequent *Salmonella* spp. survival during extended storage times of up to 28 d, after treatment has been ended.

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